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The Contents of Case 09724406

Qnum	Query	DB Name	Thesaurus	Operator	Plural
Q1	anti-cd30	USPT	ASSIGNEE	ADJ	YES
Q2	Q1 and hodgkin$2	USPT	ASSIGNEE	ADJ	YES

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DIALOG(R)File 5:Biosis Previews(R)

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The anti- CD30 monoclonal antibody SGN-30 promotes growth arrest and DNA fragmentation in vitro and affects antitumor activity in models of Hodgkin's disease.

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ABSTRACT: The leukocyte activation marker **CD30** is highly expressed on the Reed Sternberg cells of Hodgkin's disease (HD). On normal tissues, **CD30** has a restricted expression profile limited to activated T cells, activated B cells, and activated natural killer cells. This expression profile makes **CD30** an ideal target for monoclonal antibody (mAb)-based therapies of Hodgkin's disease. **CD30** mAbs have been shown to be effective in in vitro and in vivo models of hematologic malignancies such as anaplastic large cell lymphoma, yet these mAb have not been efficacious in HD models. We have found that a mAb against **CD30** , **AC10** , was able to inhibit the growth of HD cell lines in vitro. To generate a more clinically relevant molecule, the variable regions from **AC10** were cloned into an expression construct containing the human gamma1 heavy chain and kappa light chain constant regions. The resulting chimeric antibody, designated SGN-30, retained the binding and in vitro growth-inhibitory activities of the parental antibody. Treatment of HD cell lines with SGN-30 in vitro resulted in growth arrest in the G1 phase of the cell cycle and DNA fragmentation consistent with apoptosis in the HD line L540cy. Severe combined immunodeficient mouse xenograft models of disseminated HD treated with SGN-30 produced significant increases in survival. Similarly, xenograft models of localized HD demonstrated dose-dependent reduction in tumor mass in response to SGN-30 therapy. SGN-30 is being developed for the treatment of patients who have HD that is refractory to initial treatment or who have relapsed and have limited therapeutic options.

DESCRIPTORS:
MAJOR CONCEPTS: Immune System (Chemical Coordination and Homeostasis); Tumor Biology
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: **AC10** cell line (Muridae)--murine hybridoma cells; L540cy cell line (Hominidae)--human Hodgkin's disease cells; human (Hominidae)--patient

Set	Items	Description
S1	2	AC10 AND CD30
S2	1	RD (unique items)
S3	4	AC10 AND ANTIBODY
S4	3	RD (unique items)

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L2: Entry 5 of 15 File: USPT Mar 7, 2000

US-PAT-NO: 6033876
DOCUMENT-IDENTIFIER: US 6033876 A

TITLE: Anti-CD30 antibodies preventing proteolytic cleavage and release of membrane-bound CD30 antigen

DATE-ISSUED: March 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/69.6; 424/133.1, 424/138.1, 424/144.1, 424/155.1, 435/334, 435/344, 435/69.7, 435/7.23, 530/387.3, 530/387.7, 530/388.22, 530/388.8

CLAIMS:

We claim:

1. An antibody obtained from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204.

2. An antigen binding fragment of the antibody obtained from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204.

3. The antigen binding fragment of claim 2 wherein said fragment is selected from the group consisting of a Fab, a Fab', and a (Fab').sub.2 fragment.

4. A polypeptide comprising a) an antibody of claim 1, and b) a toxin conjugated thereto.

5. A polypeptide comprising a) an antigen binding fragment of claim 2, and b) a toxin conjugated thereto.

6. A chimeric antibody comprising a) the variable region of an antibody from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204 and b) the constant region of a human antibody, wherein the antibody has reduced immunogenicity in humans.

7. A humanized antibody comprising a) the six complementarity determining regions (CDR) of the antibody from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204, b) non-CDR variable regions from human variable regions, and c) the constant region of a human antibody, wherein the antibody has reduced immunogenicity in humans.

8. The cell line that is deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204.

9. A process for the production of an antibody which binds to the CD30 antigen, comprising the steps of:

a) culturing the cell line that is deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204; and

b) producing and isolating an antibody from said cell line.

10. A composition comprising the antibody of claim 1 and a pharmaceutically acceptable excipient.

11. A composition comprising the antibody fragment of claim 2 and a pharmaceutically acceptable excipient.

12. A method for the detection of Hodgkin's disease, comprising the steps of:

a) contacting a biological sample with an antibody or antibody fragment which binds to the CD30 antigen, wherein said antibody or antibody fragment is obtainable from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204, under conditions such that said antibody or antibody fragment binds to CD30 antigen present in said biological sample,

b) detecting any antibody bound to CD30 antigen, and

c) correlating antibody bound to CD30 as an indication of Hodgkin's disease.

13. A process for the production of an antibody with a reduced immunogenicity in humans comprising manipulating an antibody obtained from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204, to produce an antibody comprising a) the variable region of an antibody from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204 and b) the constant region of a human antibody.

14. A pharmaceutical composition comprising the antibody of claim 6 and a pharmaceutically acceptable excipient.

15. A pharmaceutical composition comprising the antibody of claim 7 and a pharmaceutically acceptable excipient.

16. A pharmaceutical composition comprising the polypeptide of claim 5 and a pharmaceutically acceptable excipient.

L2: Entry 5 of 15 File: USPT Mar 7, 2000

DOCUMENT-IDENTIFIER: US 6033876 A
TITLE: Anti-CD30 antibodies preventing proteolytic cleavage and release of membrane-bound CD30 antigen

Abstract Text (1):
An antibody which binds to the CD30 antigen and a) inhibits the release of sCD30 from Hodgkin's disease cells, and b) does not bind to B cell non-Hodgkin's lymphomas or plasma cells. An example of such antibodies are the antibodies secreted from hybridoma cell line DSM ACC 2204. The antibodies may be used for diagnosis, or conjugated to a toxin to produce an immunotoxin.

Brief Summary Text (1):
The invention comprises high-affinity Hodgkin's disease cell specific anti-CD30 antibodies which prevent proteolytic cleavage and release of membrane-bound CD30 antigen, a method of production and the use of said antibodies.

Brief Summary Text (3):
The CD30 activation marker was originally discovered as the Hodgkin-associated Ki-1 antigen (Schwab et al. (1982) (1)). CD30 is a membrane glycoprotein with a molecular weight of 120 kDa (Froese et al. (1987) (8)). A soluble form of the CD30 (sCD30) is released from cell membranes (Hansen et al. (1989) (2)) which is detectable in sera of Hodgkin patients (Josimovic-Alasevic et al. (1989) (3), Pfreundschuh et al. (1990) (4)) and the serum levels of the sCD30 correlate with the severity and the clinical stage of the disease (Pizzolo et al. (1990) (5)). sCD30 is a cleavage product of the cell surface-bound CD30 molecule, as it could be shown that the glycosylation pattern of CD30 and sCD30 is identical. CD30 is cleaved by a specific acting protease.

Brief Summary Text (4):
The membrane-associated CD30 antigen is regarded as a possible target for treatment of Hodgkin's-diseased patients with immunotoxins. However, the efficacy of the various antibody-toxin conjugates show rather big differences (Engert et al. (1990) (6)). Moreover, the CD30-specific monoclonal antibody (mAb) Ki-1 enhanced the release of the sCD30 from the Hodgkin-derived cell lines L428 and L540 as well as from the CD30+ non-Hodgkin's lymphoma cell line Karpas 299 (Hansen et al. (1991) (7)).

Brief Summary Text (6):
The conjugate of antibody Ki-1 with the Ricin A-chain, for instance, was a rather ineffective immunotoxin and it was concluded that this ineffectiveness was due to the rather low affinity of antibody Ki-1 (Engert et al. (1990) (6)). Two other reasons may also account for the weak toxicity of Ki-1-Ricin A-chain conjugates: a) Antibody Ki-1 enhanced the release of the sCD30 from the Hodgkin-derived cell lines L428 and L540 as well as from the CD30+ non-Hodgkin's lymphoma cell line Karpas 299 Hansen et al. (1991) (7)); b) the relatively great distance of the Ki-1 epitope from the cell membrane is also not favorable for the construction of potent immunotoxins (Press et al. (1988) (11), May et al. (1990) (12)).

Brief Summary Text (7):
At the Fourth Workshop on Leukocyte Differentiation Antigens in Vienna in February 1989, monoclonal antibodies were submitted by three different laboratories and finally characterized as belonging to the CD30 group. Co-cultivation experiments by the inventors of L540 cells with various antibodies according to the state of the art, followed by the isolation of sCD30 from culture supernatant fluids, revealed that the release of the sCD30 was most strongly increased by antibody Ki-1, and weakly enhanced by the antibody HeFi-1, whilst being more strongly inhibited by the antibody Ber-H2. However, the antibody Ber-H2 also labels a subpopulation of plasma cells (R. Schwarting et al. (1989) (10)) and G. Pallesen (9) describes, on page 411, that Ber-H2 is cross-reacting with an epitope of an unrelated antigen which is altered by formaldehyde. Therefore, in the state of the art, no anti-CD30 antibody is known which does not release sCD30 and is specific for Hodgkin and Reed-Sternberg cells.

Brief Summary Text (9):
It was therefore the object of the invention to provide new CD30-specific antibodies which do not promote the release of the sCD30, but inhibit the formation of the sCD30 instead and thus would possibly allow the formation of powerful immunotoxins.

In the present invention there is described the production and reactivity of new CD30-specific antibodies with special reference to the relative positions of the epitopes recognized by these and other established anti-CD30 antibodies on the extracellular part of the CD30 molecule. The new antibodies according to the invention exhibit a nearly complete inhibition of the formation of the sCD30 and do not bind to a considerable extent to plasma cells or B cell non-Hodgkin's lymphomas and are therefore specific for Hodgkin and Reed Stemberg cells.

Brief Summary Text (13):
a) release sCD30 from Hodgkin's disease cells to an amount of, or less than, about 10% referred to the release found without an addition of antibodies;

Brief Summary Text (14):
b) do not bind to B cell non-Hodgkin's lymphomas or plasma cells to a considerable extent.

Brief Summary Text (15):
As used herein the term "release sCD30" means the shedding of CD30 molecules from the cell surface of tumour cells. This release is reduced to a considerable extent using the antibodies of the invention compared to the shedding which is found in the case of CD30+ Hodgkin's cells in vitro und in vivo without antibodies. Release (shedding) of sCD30 can be tested according to the method described by Hansen et al. (1989) (2). When applying this method it was found that, using the antibodies according to the invention, the release of sCD30 from Hodgkin's disease cells could be reduced to 10% or less. Depending on the chasing time, antibody Ki-4 inhibited the shedding of the sCD30. Up to 16 h the shedding was nearly completely inhibited, i.e. less than 1%. Thereafter, the amount of sCD30 could increase to maximally 10% compared to that of untreated control cells.

Brief Summary Text (23):
With the antibodies according to the invention it is possible to find a great number of further antibodies which interact with CD30 in an analogous manner. Such antibodies are bindable to CD30 antigen in a manner equivalent to the antibodies according to the invention, especially Ki-4. Furthermore, these antibodies must be tested for the release of sCD30 from Hodgkin's disease cells, the antibodies and the cell lines, respectively, which release sCD30 to an amount of, or less than, 10%, referred to the release found without an addition of antibodies, are isolated, furthermore those cell lines which produce antibodies that bind to Hodgkin's disease cells but not to B cell non-Hodgkin's lymphoma or plasma cells are isolated.

Brief Summary Text (28):
a) releases sCD30 from Hodgkin's disease cells to an amount of, or less than, 10%, referred to the release found without an addition of antibodies;

Brief Summary Text (29):
b) does not bind to B cell non-Hodgkin's lymphomas or plasma cells to a considerable extent,

Brief Summary Text (30):
wherein a mammalian species is immunized with a Hodgkin's disease cell line, anti-CD30 antibody producing B cells are isolated and fused with myeloma cell lines, the fused cell lines are isolated and tested for antibody activity against Hodgkin's disease cells and the release of sCD30 from Hodgkin's disease cells, the cell lines which produce antibodies that bind to Hodgkin's disease cell lines but not to B cell non-Hodgkin's lymphomas or plasma cells to a considerable extent and release sCD30 to an amount of, or less than, 10%, referred to the release found without an addition of antibodies, are isolated, monoclonal antibodies are produced from said cell lines and isolated, preferably to substantial purity.

Brief Summary Text (33):
The present invention is also concerned with the use of an antibody according to the present invention for the diagnosis or therapy of Hodgkin's disease. It is thereby preferred to use the antibody Ki-4 secreted by the cell line DSM ACC 2204.

Brief Summary Text (34):
Since the antibodies obtained by the process according to the present invention are bindable with surface-bound CD30 molecules but inhibit sCD30 release, they are outstandingly suitable for the qualitative or quantitative detection of Hodgkin's disease. The detection thereby takes place in the known manner by means of an immunological process of determination. Processes of this type are well-known and do not need to be further explained here. The antibodies obtained according to the present invention can thereby be used as unlabelled and/or immnobilized receptors.

Brief Summary Text (37):
The present invention also provides a process for therapy of Hodgkin's disease, wherein there is administered one or a mixture of several antibodies according to the present invention, optionally together with conventional pharmaceutical carrier, adjuvant, filling or additive materials.

Brief Summary Text (40):
Since the monoclonal antibodies obtained by the process according to the present invention bind to cell surface-bound CD30 antigen, they can be used for in vivo treatment in humans. Thus, the present invention also provides a pharmaceutical composition which comprises one or more antibodies according to the present invention, optionally together with conventional pharmaceutical carrier, adjuvant, filling or additive materials. The administration of a medicament according to the present invention is useful for the treatment of Hodgkin's disease.

Brief Summary Text (41):
A suitable dosage of the antibody according to the present invention for the treatment of Hodgkin's disease is about 1 to 10 mg/kg body weight, whereby this dosage possibly is to be repeatedly administered.

Brief Summary Text (54):
Polynucleotides of the invention and recombinantly produced anti-CD30 antibodies of the invention may be prepared on the basis of the sequence data according to methods known in the art and described in Sambrook et al. (1989) (64) and Berger and Kimmel (1987) (65). Polynucleotides of the invention are preferably formed from synthetic oligonucleotides.

Brief Summary Text (63):
For therapeutic uses, a sterile composition containing a pharmacologically effective dosage of one or more antibodies according to the invention is administered to human patient for treating Hodgkin's disease. Typically, the composition will comprise a chimeric or humanized antibody which contains the CDR region of Ki-4 for reduced immunogenicity.

Detailed Description Text (5):
Several established anti-CD30 mAbs were used as reference antibodies: Ki-1 which is the prototype antibody for the CD30 antigen (Schwab et al. (1982) (1)), Ber-H2 (Scbwarting et al. (1988) (14)), Mab HRS-1 and -4 from Dr. M. Pfreundschuh, Homburg, Germany, HeFi-1 (Hecht et al. (1985) (15)), antibodies M44 and M67 (Smith et al. (1993) (16)) and antibody C10 (Bowen et al. (1993) (17)).

Detailed Description Text (7):
The Hodgkin's disease-derived cell line L540 is described in Diehl et al. (1981) (18) with respect to the isolation of CD30.sup.+ cells. L540 cells were used for immunization of BALB/c mice and for immunoprecipitation of the CD30 antigen. The immunized BALB/c spleen cells were hybridized with the non-secretor myeloma cell line X63-Ag8.653 as described (Lemke et al. (1985) (19)). The EBV-transformed B-lymphoblastoid CD30-negative cell line PDe-B-1 is described by Gatti and Leibold (1979) (20).

Detailed Description Text (11):
The specificity of the mAb was tested on human tissue specimens that had been collected during surgical operations, snap frozen in liquid nitrogen and cryopreserved at -80.degree. C. Immunoperoxidase (Schwab et al. (1982) (1)) or immunoalkaline phosphatase methods were elaborated on 5 .mu. frozen sections from nearly all normal tissue types. In addition, samples of Hodgkin's disease of mixed cellularity (n=12) and nodular sklerosis (n=8) subtypes and of large anaplastic lymphoma cases (n=5) were included. Furthermore, cases of B cell lymphoma of centroblastic type and T cell lymphoma (n=5) according to the Kiel classification were studied. Non-lymphoid neoplasias included in this study, were adenocarcinomas (n=5), squamous cell carcinomas (n=3), malignant melanomas (n=8) and malignant fibrous histiocytomas (n=3). In case of antibodies recognizing a paraffine-resistant epitope, routinely processed tissue specimens, fixed in 4% formaline and embedded in paraffine were subjected to immunoperoxidase reaction following trypsin (Sigma Chemicals, Munchen, Germany) digestion for 10 min at 37.degree. C. In parallel studies, trypsin digestion was omitted. All cases were diagnosed in the Kiel Lymphoma registry of the German Society of Pathologists by light microscopy in H&E and Giemsa stained paraffine sections. The diagnosis was further supported by immunohistochemistry with a panel of cell lineage-specific mAb (Parwaresch et al., in preparation (22)).

Detailed Description Text (15):
The experiments for determining the mutual binding inhibition of the anti-CD30 antibodies were done as described (Lemke and Hammerling (1982) (24)). The antibodies were purified from culture supernatant fluids by affinity-chromatography either with the aid of SpA-S or GaMIg covalently coupled to CNBr-activated SEPHAROSE.RTM. CL4B (highly cross-linked 4% agarose). (Pharmacia, Freiburg, Germany). The concentration of the eluted protein was determined by the method of Whitaker and Granum (1980) (25) and the content of specific antibody was calculated as described (Lemke and Hammerling (1982) (24)).

Detailed Description Text (17):
Production of anti-CD30 antibodies

Detailed Description Text (20):
The new mAbs showed a highly restricted immunohistochemical distribution pattern in human tissue and their specificity was therefore established in further studies. With respect to normal human tissue, no reactivity was encountered in tissue samples from brain, skin, lung, heart and vessels, endocrine and exocrine glands, gastrointestinal tract, hepatobiliary system, Kidney and

urogenital tract, muscles, bone, cartilage and soft tissue. Also hematopoietic cells of blood were entirely negative with these antibodies, whereas like with antibody Ki-1 a few cells in the bone marrow (Schwab et al. (1982) (1)) reacted also with antibodies Ki. In lymphoid tissues such as tonsils, lymph node and spleen, only a few lymphoid cells in the perifollicular areas showed a weak surface bound reactivity to the new antibodies. Thymus tissue was completely negative.--We also tested a large panel of permanent cell lines established from transformed human cells. The new antibodies showed an identical reactivity pattern as the established anti-CD30 antibodies Ki-1 and Ber-H2.

Detailed Description Text (21):
The immunohistochemcal analysis of a panel of non-hematopoietic human malignancies revealed that the new antibodies did not react with any of the adenocarcinomas (n=5), squamous cell carcinomas (n=3), malignant melanomas (n=8), malignant fibrous histiocytomas (n=3) and 2 cases of neurosarcomas. In case of hematopoietic neoplasias negative for CD30, no co-reactivity of these antibodies was found with acute myeloid leukemia (n=3), acute monocytic leukemia (n=3), chronic myeloid leukemia (n=3), pre-B lymphoblastoid leukemia (n=2), thymic lymphoblastoid leukemia (n=1), malignant T-lymphoma (n=5) and malignant B-lymphoma (n=3). In a panel of 18 cases of CD30-positive human lymphoma types, the monoclonal antibodies were regularly positive in a complete congruence with the reactivity of the established anti-CD30 antibodies Ki-1 and Ber-H2. The characteristic Sternberg-Reed and Hodgkin cells in nodular sklerosis or mixed cellularity type of Hodgkinis disease showed a variable reactivity with all six antibodies. In cases of Ki-1 positive large cell anaplastic lymphoma, where over 60% of the tumour cells expressed CD30, the new antibodies revealed a comparable frequency of positivity.

Detailed Description Text (22):
Properties of the anti-CD30 antibodies

Detailed Description Text (23):
In addition, the specificity of the new anti-CD30 antibodies was tested by immunoprecipitation of the recognized molecules from CD30 positive and for control purposes from negative cell lines. The results of these experiments are summarized in Table I. The antibodies allowed the isolation from positive, but not from CD30 negative cell lines of two molecules of 90 and 120 kDa respectively which most likely were identical to the 90 kDa precursor and the 120 kDa mature membrane form of the CD30 antigen (Hansen et al. (1989) (2)). The identity of the 90/120 kDa molecules isolated by the antibodies with the CD30 activation marker was confirmed by sequential immunoprecipitation, using antibodies Ki-1 and Ber-H2 as CD30-specific reference antibodies.

Detailed Description Text (24):
Binding inhibition experiments with anti-CD30 monoclonal antibodies

Detailed Description Text (26):
The binding of radio-iodinated mAb Ki-1 to CD30+ Hodgkin's disease-derived L540 target cells was competed by other CD30-specific mAbs. For this, iodine-125-labeled mAb Ki-1 (0.2 .mu.g/ml) was incubated with 2.times.10.sup.5 L540 cells in the presence of different concentrations of non-labeled mAb as inhibitor in a total volume of 60 .mu.l for 90 min at RT. In the experiment, the 100% binding value without inhibitor corresponds to 4,540 cpm bound. The binding of the Ki-1 mAb was equally well inhibited by non-labeled mAb Ki-1 and not influenced by mAb Ki-4, while it was enhanced by Ber-H2. From such binding inhibition curves, binding inhibition factors could be estimated that indicate how much more of the heterologous competitor had to be used to obtain 50% inhibition compared with the amount of cold homologous antibody which yielded 50% inhibition. These data are summarized in Table II. Lately, the mAb M44 and M67 (Smith et al. (1993) (16)) and mAb C10 (Bowen et al. (1993) (17)) were included in this study. The amounts of purified antibodies M44 and M67 were sufficient to use them as radio-labeled indicators and mAb C10 could be used as cold competitor.

Detailed Description Text (29):
Anti-CD30 antibody Ki-1 enhances the shedding of the CD30 membrane antigen (Hansen et al. (1991) (7)). Since this phenomenon would counteract the toxic effects of immunotoxins, it was tested whether the anti-CD30 Mabs according to the invention influenced the shedding of this activation marker as described (Hansen et al. (1989) (2)). For this, L540 cells were pulse-labeled with S-35-methionine for 10 min, washed and resuspended in fresh medium. Then, aliquots of 2.times.10.sup.5 cells were cultured for a chase period of 16 h either without antibody or mAb Ki-1, Ber-H2, Ki-4 and HeFi-1. The sCD30 was isolated as described in example 1, analyzed by SDS-PAGE (7.5-15% gradient gels under reducing conditions) and visualized by autoradiography. Compared to the negative control cultures without antibody, the release of the sCD30 was most strongly enhanced by Ki-1 and enhanced to different degrees by M44 and HeFi-1. In contrast, mAb Ber-H2 and Ki-4 clearly inhibited the release of sCD30 from L540 cells. The reduction of the sCD30 by mAb Ki-4 seemed reproducibly slightly stronger than that induced by Ber-H2.

Detailed Description Text (32):
CD30-positive cells, e.g. Hodgkin-analogous L540 cells, were pulse-labelled with S-35-methionine for 10 min., followed by the removal of unincorporated material and the addition of an excess of cold methionine in fresh RPMI 1640 medium. After

different periods of chasing time, the CD30 antigen was isolated either from the cells or from the supernatant fluids of those cell samples by immunoprecipitation with the aid of said anti-CD30 antibodies. The immune complexes were isolated by affinity chromatography on staphylococcal protein A-SEPHAROSE.RTM. CL-4B and the antigenic molecules were analyzed by autoradiography after separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The quantity of the isolated sCD30 was estimated from the intensity of the labelled bands or such bands were excised from the gels and the amount of radioactivity was measured by liquid scintillation counting.

Detailed Description Paragraph Table (1):
TABLE I _____ Properties of monoclonal anti-CD30 antibody Ki-4 Reaction with antigen Designation detection of antigen of anti-CD30 Protein A cryo- paraffin-embedded sections antibodies Isotype binding M.sub.r sCD30.sup.a) sections without plus trypsin _____ Ki-4 .gamma.1, .kappa. -- 90/120.sup.b) + + + + _____ .sup.a) It was tested whether the antibody could isolate the soluble form of the CD30 (sCD30) from culture supernatant fluids of Hodgkin's diseasederived L540 cells which had been labelled with .sup.35 Smethione. .sup.b) Numbers indicate the molecular weights of the immunoprecipitated different forms of the CD30.

Detailed Description Paragraph Table (2):
TABLE II _____ Binding inhibition of iodine-125-labelled anti-CD30 antibodies by non-radiolabelled antibodies.sup.a Iodine-125-labelled anti-CD30 antibodies Cluster A Cluster B Cluster C Inhibitor.sup.b Ki-4 Ber-H2 Ki-1 M67 M44 HeFi-1 _____ Ki-4 1.sup.c 3.sup.d - - - - Ber-H2 0.5 1 -.sup.e - - - HSR-1 - +.sup.f - - - - HSR-4 + + - ..sup.h . . Ki-1 - - 1 13 - - M67 . . . 1 . . M44 . . . . 1 . HeFi-1 - - - - 3.9 1 C10 . . . . +++.sup.i +++.sup.i _____ .sup.a) Assay was done with Hodgkin's diseasederived CD30.sup.+ L540 cells. .sup.b) Antibodies HSR1 and 4 were only available in low amounts as nonpurified culture supernatant fluids. The amounts of purified M44 and M67 allowed the application as radiolabelled indicator, but not as nonlabelled competitors. .sup.c) The amount of homologous antibody that gave 50% inhibition is set 1. .sup.d) Numbers indicate the factor by which the homologous binding inhibition value 50% has to be increased to obtain 50% inhibition with th heterologous antibody. .sup.e) No significant inhibition. .sup.f) Significant inhibition was observed at high concentrations of heterologous antibody, but 50% inhibition was not reached. .sup.h). = not tested. .sup.i) +/+++ = Antibody C10containing ascites fluid induced 45% inhibition of radiolabelled antibody Ki3 (+) and complete inhibition of antibodies M44 and HeFi1 (+++).

Other Reference Publication (1):
Linnartz, et al., Annals of Hematology, vol. 67 (suppl.) (Oct. 10-13) 1993, p. A77 XP 000567763, abstract 298, "Development of new ricin A-chain immunotoxins for the treatment of hodgkins's disease using high-affinity mooclonal antibodies against the CD-30 antigen".

Other Reference Publication (3):
Falini, et al., British Journal of Hematology, vol. 82, No. 1 (1 992), pp, 38-45, "In vivo targeting of Hodgkin and Reed-Sternberg cells of Hodgkin's disease with monoclonal antibody Ber-H2 (CD30): Immunohistological evidence".

Other Reference Publication (4):
Horn-Lohrens, et al., International Journal of Cancer, vol. 60, No. 4 (Feb. 8) 1995, pp. 539-544, "Shedding of the soluble form of CD30 from the Hodgkin-analogous cell line L540 is strongly inhibited by a new CD30-specific antibody (Ki-4)".

Other Reference Publication (5):
Schnell, et al., International Journal of Cancer, vol. 63, No. 2 (Oct. 9) 1995, pp. 238-244, "Development of new ricin A-chain immunotoxins with potent anti-tumor effects against human Hodgkin cells in vitro and disseminated Hodgkin tumors in SCID mice using high-affinity monoclonal antibodies directed against the CD30 antigen".

CLAIMS:

12. A method for the detection of Hodgkin's disease, comprising the steps of:

a) contacting a biological sample with an antibody or antibody fragment which binds to the CD30 antigen, wherein said antibody or antibody fragment is obtainable from the cell line deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) under accession number ACC 2204, under conditions such that said antibody or antibody fragment binds to CD30 antigen present in said biological sample,

b) detecting any antibody bound to CD30 antigen, and

c) correlating antibody bound to CD30 as an indication of Hodgkin's disease.